MODULE 23: USE OF ANTIBIOTICS IN ANIMALS

NATIONAL VETERINARY ACCREDITATION PROGRAM

United States Department of Agriculture • Animal and Plant Health Inspection Service • Veterinary Services

Approved as one unit of supplemental training for participants in USDA's National Veterinary Accreditation Program

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National Veterinary Accreditation Program

Use of Antibiotics in Animals

This informational module has been approved expressly to serve as one unit of supplemental training for participants in USDA's National Veterinary Accreditation Program. This module was developed in consultation with the American Veterinary Medical Association, U.S. Centers for Disease Control and Prevention, U.S. Food and Drug Administration's Center for Veterinary Medicine, Food Animal Residue Avoidance Databank, U.S. Armed Forces Health Surveillance Center, Iowa State University College of Veterinary Medicine, University of Nebraska Great Plains Veterinary Educational Center, Oklahoma State University Center for Veterinary Health Sciences, and Texas A&M University College of Veterinary Medicine. Information in the module does not supersede the regulations. For the most up-to-date regulations and standards, please refer to the Code of Federal Regulations. References and additional information are available at the end of this module.

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Use of Antibiotics in Animals

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Introduction

One of the primary roles of many accredited veterinarians is to identify, treat, control, and prevent disease in animals. Many of these diseases are a result of a bacterium and require antibiotic administration as a means of treatment to relieve animal suffering and reduce pathogen load in animals destined for human consumption. Deciding which antibiotic to use can be complicated in some cases. Veterinarians utilize their problem solving skills, clinical training, and information gained through continuing education to arrive at the best possible option to treat their patients. Increasing awareness of the diagnostic tools, regulatory issues, and antibiotic resistance debate led to the development of this educational module.

After completion of this module you should be able to:

- Evaluate various parameters when selecting antibiotics for use in animals.
- Describe the benefits and limitations of various antibiotic susceptibility testing options.
- Locate and interpret antibiotic labels for the purposes of informed therapeutic decision-making.
- List the agencies involved in regulating antibiotics and monitoring antibiotic resistance and residues.
- Apply the key components of the Animal Medicinal Drug Use Clarification Act (AMDUCA) to making decisions about antibiotics.
- Locate information to assist in making decisions on antibiotic use in animals.

Completion of this module is estimated to take 60 minutes but will vary depending on your familiarity with the material.

Antibiotic Selection

Antibiotic selection should be based upon the following key factors:

- Characteristics of suspected or confirmed bacterial organism(s)
- Prevalence of antibiotic resistance mechanisms in the suspected or confirmed bacterial organism(s)
- Ability to reach site of infection at an effective concentration
- Animal species and their potential for human consumption
- Dose, frequency, duration of therapy, and route of administration of available antibiotic formulations

Antibiotics are defined as a subset of antimicrobial agents produced by a mold or a bacterium that slows the growth of, or kills, other microbes (e.g., streptomycin and penicillin). Antimicrobial agent is a more overarching term that includes the drugs, chemicals, or other substances that slow the growth of, or kill, microbes (e.g., antibiotics, antivirals, antifungals, and antiparisitic drugs). This module focuses upon antibiotic use; however, the principles presented are also applicable to all antimicrobial agents.

Source:

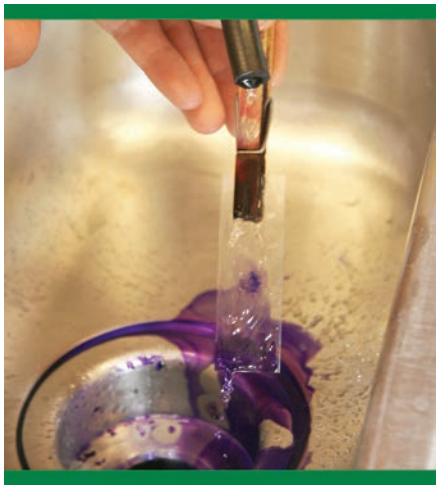
- *Centers for Disease Control and Prevention. Antibiotic/Antimicrobial Resistance: Glossary. Accessed February 25, 2012 at http://www.cdc.gov/drugresistance/glossary.html#antibiotic*

Characteristics of Suspected or Confirmed Bacterial Organism

One of the first steps in selecting an appropriate antibiotic is to know about the target bacteria. This can be accomplished through culture, which is the growth of bacterial organisms in media. Samples from the site of infection of an individual animal or a collection of samples from a herd or flock can be used to determine the characteristics of the bacterial organism of concern.

Culture will also determine if the target bacterium is aerobic, microaerophilic, or anaerobic (facultative or obligate), which is also important when selecting an antibiotic. For example, uptake of aminoglycosides into a bacterium uses an oxygen-dependent mechanism. Aminoglycosides are less active against anaerobes, but can be particularly active against aerobic bacteria.

Aerobes like *Streptococcus* spp., *Staphylococcus* spp., *Mycobacterium tuberculosis*, and *Pseudomonas aeruginosa* produce energy using oxygen. Microaerophiles like *Borrelia burgdorferi* and *Campylobacter* spp. need oxygen to live but at lower than atmospheric levels. Anaerobes produce energy without the use of oxygen. Facultative anaerobes make energy using oxygen when it is available, but are capable of surviving when oxygen is not present. Common facultative anaerobes include *Escherichia coli* and *Klebsiella pneumoniae*. Obligate anaerobes are sensitive to oxygen; they either do not grow or die if oxygen is present. Common obligate anaerobic bacteria include *Clostridium tetani* and *Fusobacterium necrophorum*.

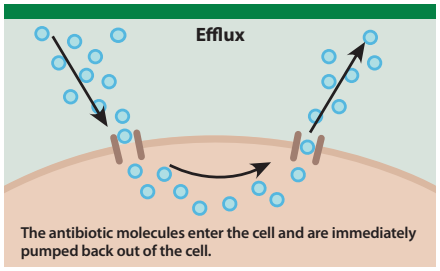


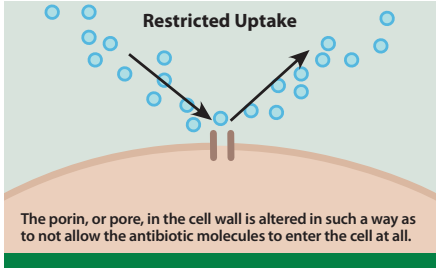
Gram staining* is another component in bacterial classification. Common Gram-positive bacteria include *Streptococcus spp.*, *Staphylococcus spp.*, *Clostridium spp.*, *Enterococcus spp.*, *Corynebacterium spp.*, and *Listeria monocytogenes*. Common Gram-negative bacteria include *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Klebsiella pneumoniae*, *Enterobacter* spp., and *Haemophilus parasuis*.

*Gram staining is a laboratory technique using a series of different aqueous solutions that adhere to the peptidoglycan comprising the bacterial cell wall (purple = Gram-positive and pink = Gram-negative). Not all bacteria will produce Gram stain results and additional diagnostics are needed to classify them.

Certain antibiotics are most effective against one group with little activity against the other. For example, some macrolides cannot penetrate the cell walls of Gram-negative organisms in the family *Enterobacteriaceae*.

Some antibiotics are clinically effective against Gram-positive **and** Gram-negative bacteria. An example would be amoxicillin/clavulanic acid. Activity against multiple classes of bacteria is not always desirable. If the target bacterium is known, it is better to target treatment more specifically against the offending microbe in order to reduce the risk of resistance emerging in other bacterial groups. Broad spectrum antibiotics are better reserved for critically ill animals when there is not time to gather the information on the bacterial classification or if there are multiple bacteria involved in an infection.





Prevalence of Antibiotic Resistance Mechanisms

Prevalence of antibiotic resistance mechanisms in the suspected or confirmed microorganism is also important to consider. Organisms can resist the action of antibiotic agents by different mechanisms:

1. Efflux,
2. Drug inactivation,
3. Changes in targets, and
4. Restricted uptake.

For example, numerous bacteria have nonspecific efflux pumps* including *Mycobacterium* spp., *Escherichia* spp., and *Pseudomonas* spp. Many *Staphylococcus* spp. have developed an enzyme, penicillinase, which makes them resistant to penicillins. A similar enzyme, cephalosporinase,

can produce resistance to all cephalosporins, penicillins, monobactams, and cephamycins (drug inactivation). *Campylobacter* spp. quickly develop resistance to fluoroquinolones through numerous point mutations in the bacteria's DNA gyrase** gene that decreases the antibiotic's affinity for the gyrase (changes in targets). *Salmonella enterica* strains can be resistant to multiple antibiotics due to acquisition of plasmids***. Further, *Mycobacterium* spp. have cell walls that contain a high lipid content, which not only makes them resistant to killing in macrophages but also restricts uptake and penetration of antibiotic agents.

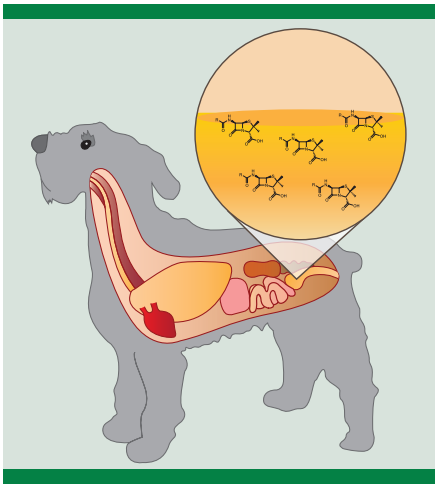
Sources:

- *Carattoli A. Plasmid-Mediated Antimicrobial Resistance in Salmonella enterica.* Current Issues in Molecular Biology *2003, 5: 113-122.*
- *Lambert, PA. Cellular impermeability and uptake of biocides and antibiotics in Gram-positive bacteria and mycobacteria.* Journal of Applied Microbiology *Symposium Supplement 2002, 92, 46S–54S.*
- *Lambert, PA. Mechanisms of antibiotic resistance in Pseudomonas aeruginosa. J R Soc Med 2002; 95 (Suppl. 41): 22-26.*
- *Nelson, M. Modulation of Antibiotic Efflux Bacteria.* Current Medicinal Chemistry *2002, 1, 35-54.*

*Efflux pumps are transport proteins involved in the extrusion of substrates (including virtually all classes of clinically relevant antibiotics). *Source: Webber, MA, Piddock, LJV. The importance of efflux pumps in bacterial antibiotic resistance.* Journal of Antimicrobial Chemotherapy. *2003. 51(1) 9-11.*

**DNA gyrase is an essential bacterial enzyme that catalyzes the ATP-dependent negative super-coiling of double-stranded closed-circular DNA. *Source: Reece RJ, Maxwell A. DNA gyrase: structure and function.* Critical Reviews in Biochemistry and Molecular Biology *1991; 26(3-4):335-75.*

***Plasmids are self-replicating double-stranded circles of DNA. *Source: Carattoli A.* Plasmid-Mediated Antimicrobial Resistance in *Salmonella enterica*. Current Issues in Molecular Biology *l. (2003) 5:113-122*



Site of Infection

Respiratory diseases, urinary tract infections, septicemia, and mastitis are commonly treated conditions in animals. Many antibiotics have particular characteristics that allow them to target the site of infection.

- Macrolides are transported to the site of infection by neutrophils, which is the body's primary line of defense in respiratory infections.
- Penicillins are found at high concentrations in the urine and are therefore commonly used in small animal urinary tract infections.
- Sulfonamides are well absorbed and widely distributed to tissue so are often used for local and systemic infections like septicemia.
- Mastitis can be difficult to treat due to a number of factors:
  - Some systemic antibiotics may not be effectively taken up by leukocytes for transportation to the site of infection (i.e., the mammary gland).
  - The infecting microbe may not be susceptible to drugs approved for intramammary use.

Sources:

- *Ahrens, F.A. and Martin, R.J. "Antimicrobial Drugs."* Handbook of Veterinary Pharmacology. *Hsu, W.H. Ames: Wiley-Blackwell, 2008. pp 347-377.*
- *Morin, D.E. "Mammary Gland Health and Disorders."* Large Animal Internal Medicine. *B.P. Smith. St. Louis: Mosby Elsevier, 4th Edition, 2008. pp. 1112-1143.*

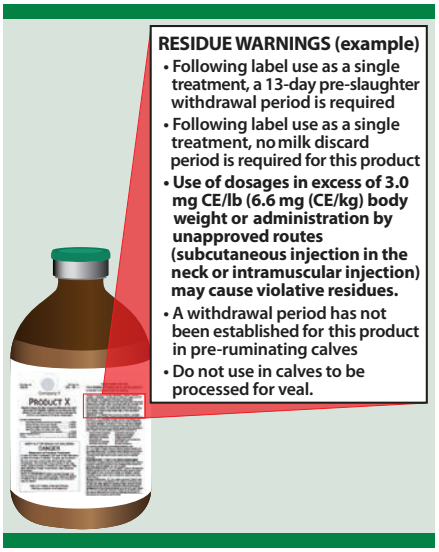
Animal Species and their Potential for Human Consumption

The animal species needing treatment and their potential for human consumption also impacts antibiotic selection. Only certain antibiotics are approved for use in food-producing animals* and meat, milk, and egg withdrawal times** must be met. Withdrawal time is the minimum length of time between drug administration and introduction of the animal or animal products into the human food chain. Under the Federal Meat Inspection Act and the Poultry Products Inspection Act, any raw meat or poultry shown to contain residues above established tolerance levels is considered adulterated and must be condemned. For example, procaine penicillin G administered intramuscularly

has a withdrawal time of 10-14 days in cattle, nine days in sheep, seven days in pigs, and a 48-hour milk discard time. This antibiotic is not labeled for pre-ruminating calves (those still consuming milk to meet their nutritional needs including those raised for veal). Introduction of the treated animal or its milk into the food chain prior to this time would be illegal.

Source:

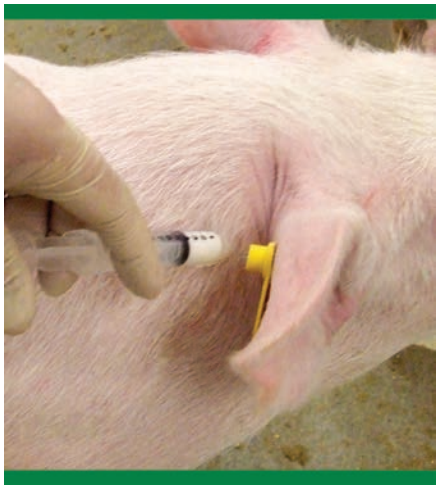
- *Fact Sheets: Poultry Preparation. Accessed December 24, 2011 at http://www.fsis.usda.gov/factsheets/turkey_from_farm_to_table/index.asp*



*Food-producing animals are those animals used in the production of food for humans, including in common usage, the species and breeds that also supply fiber and hides for human use. In the United States this includes cattle, swine, poultry, sheep, and goats. *Source: Blood, D. C., Virginia P. Studdert, and Clive C. Gay.* Saunders Comprehensive Veterinary Dictionary. *3rd ed. Edinburgh: Elsevier Saunders, 2007.*

**Withdrawal times are determined by a multistage scientific process. First, the No Observed (Adverse) Effect Level (NO(A)EL) is established by identifying the highest dose that does not cause adverse effects in the animal. Next, the Acceptable Daily Intake (ADI) is established by dividing the NO(A)EL by an 'uncertainty' value. Third, a Safe Concentration is established by multiplying the ADI x 60 kg and dividing this number by a consumption factor. Next, a marker residue is identified which can reliably predict drug depletion in edible tissues and can be measured using existing analytical techniques. Finally, the rate at which the marker residue decreases (after treatment) below the safe concentration in edible tissues and foodstuffs in measured and the withdrawal period calculated. *Source: FDA Center for Veterinary Medicine's Procedure for Setting Tolerances. Accessed May 21, 2012 at www.fda.gov/ohrms/dockets/ac/02/slides/3816s1_03_Fried.ppt*

Dose, Frequency, Duration of Therapy, and Route of Administration



The dose (milligrams of drug per kilogram of animal's body weight), frequency, duration of therapy, and route of administration are also important to consider when selecting an antibiotic. The therapeutic dose necessary to reach the target bacteria is an important consideration in selecting an antibiotic. The amount of drug required to reach a therapeutic dose may not be tolerated by the animal and therefore, alternatives need to be considered.

The frequency at which a drug is administered is often based upon certain characteristics of that drug and varies greatly between certain antibiotics. Some antibiotics need to be administered three times a day to reach therapeutic levels for the target organism in the body while others may only require two doses in a twenty-eight day period. Route of administration also varies and may be intramuscular, subcutaneous, or intravascular injection, intranasal, intramammary, topical, or oral. Animal caretakers should be trained by veterinarians when administering antibiotics regarding the proper dose, route, frequency, and duration of administration. Awareness of quality assurance programs in cattle (beef and dairy) and swine is also important.

For more information on properly administering antibiotics in food-producing animals, see:

- Beef Quality Assurance National Manual at: *http://www.bqa.org/CMDocs/bqa/NationalManual.pdf*
- Dairy Animal Care and Quality Assurance Manual at: *http://www.bqa.org/CMDocs/bqa/DairyBQAManual.pdf*
- Pork Quality Assurance guidance at: *http://www.pork.org/filelibrary/PQAPlus/PQAPlusEdBook.pdf*

Knowledge Review #1

Various parameters should be considered to assure proper antibiotic selection and optimize success. Select ALL that apply.

- **A.** Site of infection
- **B.** Animal species
- **C.** Culture results
- **D.** Aerobic stability of the organism
- **E.** Dose, frequency, duration, and route of administration
- **F.** Gram stain results

Answers are found in the appendix.

Antibiotic Susceptibility Testing

Performing an antibiotic susceptibility* test on a pure culture of the bacterial organism should be done when 1) susceptibility cannot be predicted solely based on the identified organism or 2) the animal may have an adverse reaction to the antibiotic of choice. Bacterial pathogens in animals and humans change over time which is another reason why culture and susceptibility testing is essential for selecting an efficacious antibiotic.

There are multiple *in vitro* methods available to determine the antibiotic susceptibility of a target organism and they are described on subsequent pages. Interpretation of the results is the responsibility of the veterinarian.

*Antibiotic susceptibility testing was previously referred to as 'sensitivity' testing but the principles remain the same: determining the *in vivo* success or failure of an antibiotic for an infection due to the isolate tested *in vitro* for that animal species. Source: 2008 Clinical and Laboratory Standards Institute (CLSI) document M31-A3

The methods to determine the susceptibility of an organism to an antibiotic include disk diffusion (Kirby-Bauer), broth microdilution, and antibiotic concentration gradient.

Disk Diffusion

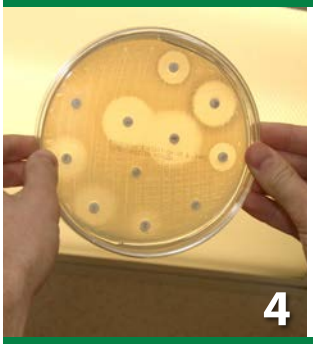
The disk diffusion method involves:

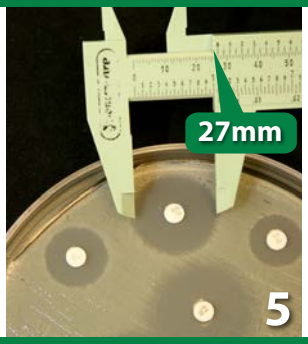
1. Seeding a growth medium (nutrient agar) with the bacterial isolate that has been diluted to a standard concentration. *Escherichia coli* was used in this example.
2. Dispensing several (up to 12) commercially prepared filter paper disks, each impregnated with a standard concentration of a different antibiotic, onto the agar surface.
3. Incubating overnight.

4. Observing bacterial growth around each disk. The antibiotic disks with growth immediately surrounding them are considered resistant.
5. Measuring in millimeters (mm) the clear zone around an antibiotic disk (area around the disc that has no growth is referred to as the inhibition zone diameter or IZD). In this example, the IZD for ceftiofur measures 27mm.
6. Comparing the IZD* to a standard interpretation chart** used to classify the bacterial isolate as susceptible (S), intermediately susceptible (I), or resistant (R). Some of the example *E. coli* results are shown.

*IZD will vary depending on the antibiotic – the largest IZD does not always predict that the bacterium is 'most susceptible' to that antibiotic.

**The chart indicates that two of the values are from approved human standards, as opposed to veterinary standards, in the Clinical and Laboratory Standards Institute (CLSI) publication M31-A2. This publication provides veterinary diagnostic labs with the currently recommended information on quality control, interpretation, and techniques for antibiotic susceptibility testing. The CLSI is an international, interdisciplinary, non-profit, standards-developing, and educational organization that promotes the development and use of voluntary consensus standards and guidelines within all specialties of the health care community.





Susceptibility Disks & Interpretive Zone Diameter Sizes
(in Millimeters)

Regular Disks	R (<or=)	I	S (>or=)	*E. coli*
Ampicillin (AM)	64	14-16	17	21 S
Ceftiofur (XNL)	8	18-20	21	27 S
Cephalothin (CF) *	32	15-17	18	17 I
Clindamycin (CC)	8	15-20	21	0 R
Erythromycin (E) *	13	14-22	23	12 R

* Values from approved human standards in CLSI M31-A2

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Disk Diffusion Advantages and Limitations

Advantages of the disk diffusion method include:

- Cost (least expensive option)
- Ease of use
- Easily interpreted categorical results
- Easily customizable by selecting specific antibiotic impregnated disks
- Sophisticated equipment is not required

Limitations of the disk diffusion method include:

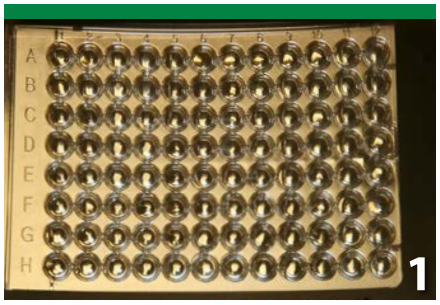
- Minimum Inhibitory Concentration (MIC) measurements are not determined from this qualitative test
- Does not provide enough information to calculate a dose
- Interpretation of inhibition may be variable (human variation)
- Not all antibiotics are available to be tested
- Fewer Clinical and Laboratory Standards Institute (CLSI)-approved standards compared to MIC
- Breakpoints are not available to predict clinical success with bacteria causing diarrhea
- Difficult to determine susceptibility of fastidious or slow-growing organisms

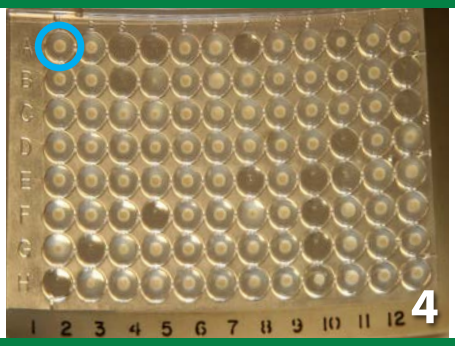
Broth Microdilution

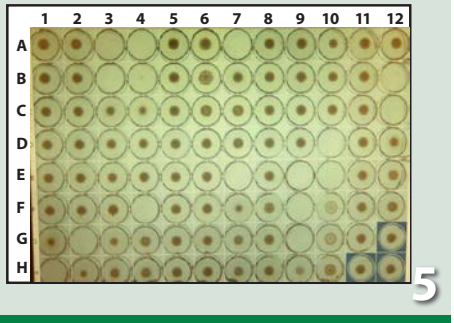
The broth microdilution method is quantitative and results in a minimum inhibitory concentration or MIC (recorded as milligrams per liter – mg/L or micrograms per milliliter – µg/ml) for a particular bacterial isolate. In this method, the isolate is grown in wells with differing (generally serially doubling) concentrations of antibiotic agents. The lowest concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the MIC.

The steps in the broth microdilution method are described on page 7.

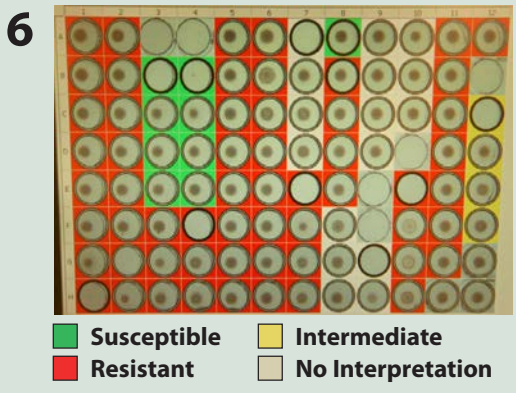
1. Begin with a sterile plastic plate with differing (generally serially doubling) concentrations of antibiotic agents in wells. There are different commercially available plates for different combinations of antibiotics. As pictured, ceftiofur at 0.25 µg/ml starts in well F1 and doubles concentration in each well up to 8.0 µg/ml in A1.
2. Each well is inoculated with a standardized number of the bacterial isolate. In this example, it was *Bordetella bronchiseptica* from pig lungs.
3. The plastic microtiter plate is incubated overnight.
4. Each well in the microtiter plate is evaluated for growth – this can be done visually as shown here or with computer software (step 5). Well A1 still had growth in this example (circled) so it will be reported as ceftiofur greater than (>) 8.0 µg/ml. Overall, the concentration of antibiotics tested allowed growth in many wells.
5. The lowest antibiotic concentration at which the isolate is completely inhibited (as evidenced by the absence of visible bacterial growth) is recorded as the MIC. Chlortetracycline was tested in column 3, rows A-E and growth was inhibited at row B, which corresponds with 4.0 µg/ml.

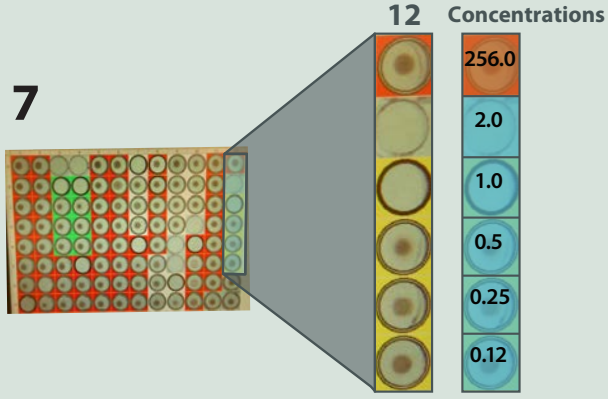




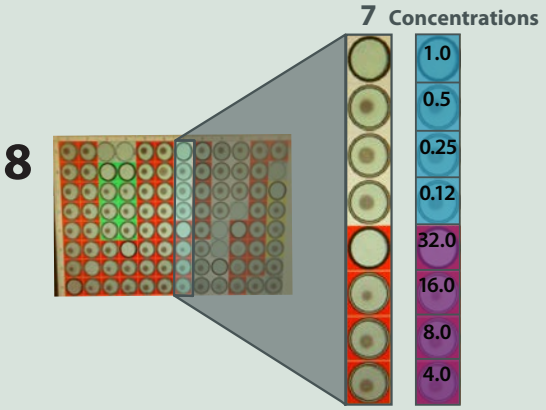


6. Based on the organism and antibiotic concentrations evaluated, each MIC value is also qualified as Susceptible (S), Intermediately susceptible (I), or Resistant (R). No Interpretation (NI) means no information is available based on the antimicrobial, organism, species, and tissue combination.
7. Column 12 is enlarged to show one well of sulfadimethoxine and enrofloxacin and the concentrations tested. Sulfadimethoxine is reported as Resistant at 256 µg/ml and enrofloxacin is Intermediate at 1.0 µg/ml.





8. Column 7 is enlarged to show two drugs (top: danofloxacin; bottom: neomycin). Danofloxacin is reported as No Interpretation despite inhibition at 1.0 µg/ml. Neomycin is Resistant at 32.0 µg/ml.
9. For demonstration purposes, only a subset of the antibiotics tested are reported in table format. Keep in mind that *in vitro* results do not represent therapeutic recommendations; interpretation of the report is up to the submitting veterinarian. Just because the lab reports a result does not mean that all antibiotics can be used in all food-producing animals (e.g., fluoroquinolones).



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Source: Swine lung
Organism: *Bordetella bronchiseptica*

Antibiotic	µg/ml
Ceftiofur	> 8.0 R
Chlortetracycline	4.0 S
Danofloxacin	1.0 NI
Enrofloxacin	1.0 I
Neomycin	32.0 R
Oxytetracycline	4.0 S
Sulfadimethoxine	> 256 R

BROTH MICRODILUTION ADVANTAGES AND LIMITATIONS

Having an MIC allows a veterinarian to modify an antibiotic regimen appropriate for a specific disease, antibiotic, and patient as described in the following sections.

Other advantages to broth microdilution method include:

- Ability to test susceptibility of multiple antibiotics at the same time
- Ease of use with commercially-prepared microtiter trays
- Rapid results with automated methods

Limitations of the broth microdilution method include:

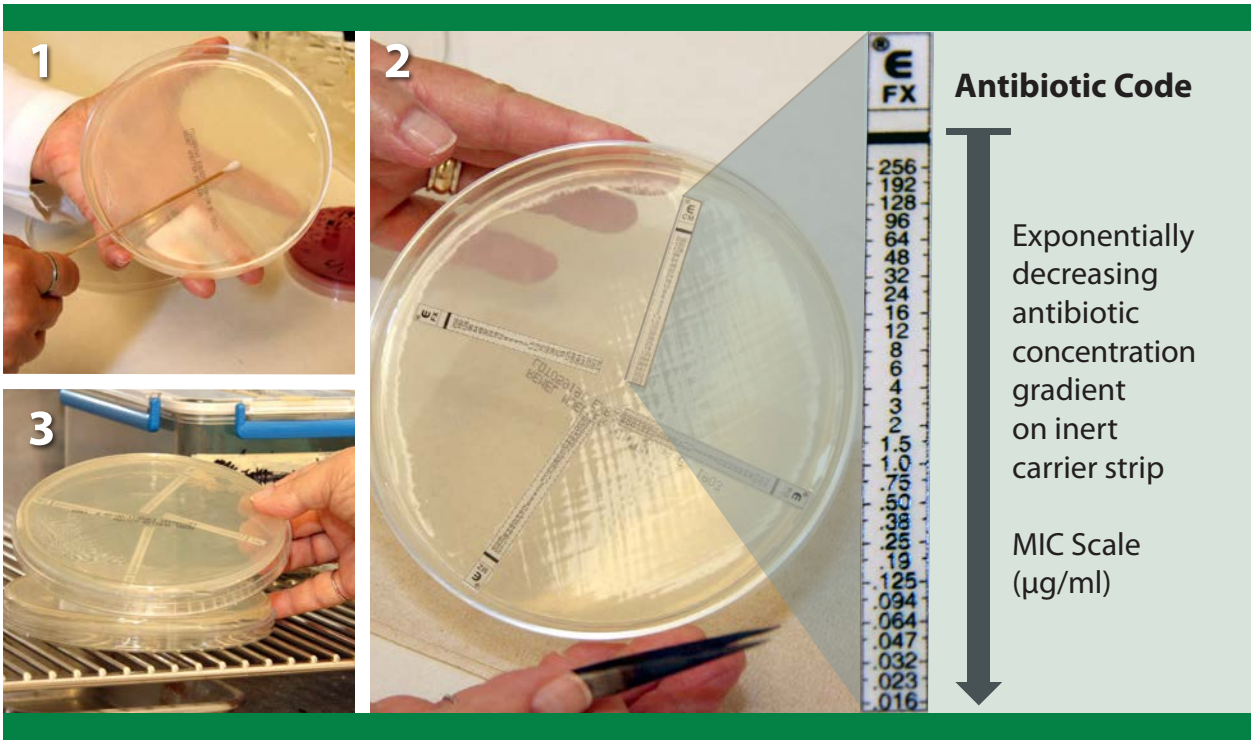
- Cost (more expensive than disk diffusion)
- Cannot customize antibiotics when using commercially-prepared kits
- Size constraints of trays limit the number of antibiotic concentrations that can be tested
- Exact MIC may be outside of the range tested (reported as 'greater than X µg/ml' rather than a precise concentration)
- Only one anaerobic bacteria (*Bacteroides*) has been laboratory confirmed to produce reliable results

ANTIBIOTIC CONCENTRATION GRADIENT

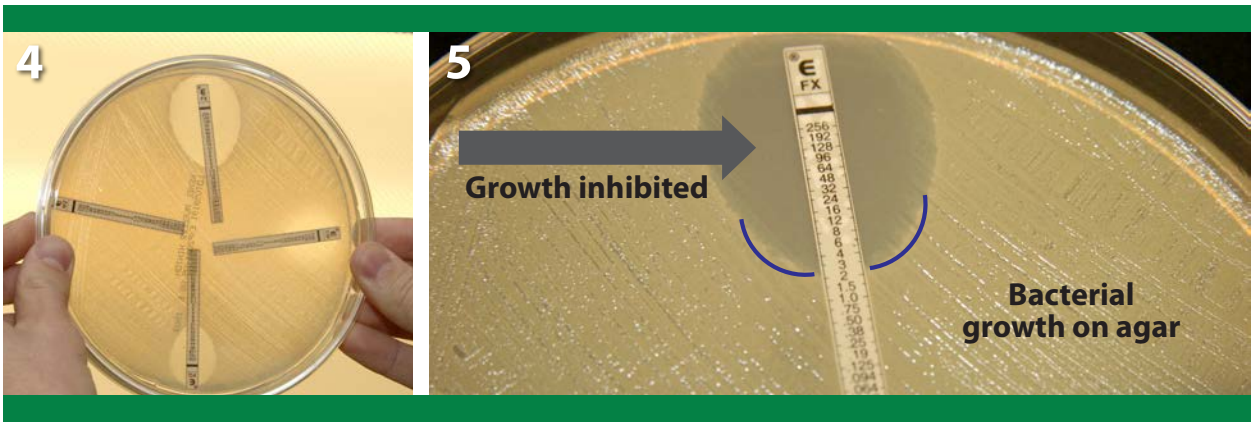
A relatively new susceptibility test, the Epsilometer test (E-test) combines the ease of disk diffusion with the quantitative benefits of broth microdilution.

The antibiotic concentration gradient method involves:

1. Preparing solid media agar with a standardized concentration of bacteria. *Esherichia coli* is used in this example.
2. Placing antibiotic impregnated plastic E-test strips with a concentration gradient on the underside (labeled the 'up' side) on the agar.
3. Incubating the agar plate overnight.



4. Observing bacterial growth around each disk. The antibiotic strips with growth immediately surrounding them are considered resistant.
5. Observing the zones of inhibition. The zones of inhibition are elliptical and intersect the plastic strip. The MIC and susceptibility is interpreted as the first value fully <u>within</u> the zone of inhibition. In this example, the inhibition line intersects the cefoxitin strip at 2 µg/ml (indicating there is still growth at 2ug of cefoxitin) so the MIC would be reported as 3 µg/ml (the first value fully within the inhibition zone – thus the *minimum* inhibitory concentration of cefoxitin required to stop growth of *E. coli* in this example).



6. Reporting the results. For the *E. coli* used in this example, the results for each of the E-test strips are reported with their MIC value and, using a standard interpretation chart, classified as Susceptible (S), Intermediately susceptible (I), or Resistant (R). The values for this example are as follows:
Cefoxitin = 3 µg/ml and is reported as Susceptible; Metronidazole > 256 µg/ml and is Resistant;
Clindamycin = 64 µg/ml Resistant; and Penicillin = 24 µg/ml, also Resistant.

6

MIC µg/ml				
Antimicrobial	**Resistant > or =**	**Intermediate**	**Susceptible < or =**	***E. coli***
Cefoxitin (FX)	64	32	16	3 S
Clindamycin (CM)	8	4	2	64 R
Metronidazole (MZ)	32	16	8	>256 R
Penicillin G (PG)	8	4	2	24 R

ANTIBIOTIC CONCENTRATION GRADIENT ADVANTAGES AND LIMITATIONS

While easy to perform, it is the most expensive susceptibility test available as it only tests one bacterium and a few antibiotics per plate. Also, there are not as many antibiotic strips applicable to veterinary medicine available as compared to disk diffusion. However, it provides an exact MIC for the bacterium and works well for fastidious bacteria and anaerobes.

Sources:

- *Michigan State University. Antimicrobial Resistance Learning Site for Veterinary Students. Examples of Antibiotic Sensitivity Testing Methods. Accessed December 15, 2011 at http://amrls.cvm.msu.edu/microbiology/detecting-antimicrobial-resistance/test-methods/examples-of-antibiotic-sensitivity-tesing-methods.*
- *Antimicrobial Therapy: General Principles. Accessed January 25, 2012 at http://cpharm.vetmed.vt.edu/vm8784/ANTIMICROBIALS/principles.htm*
- *Lee, M.* Basic Skills in Interpreting Laboratory Data. *Chapter 16 Infectious Diseases,* American Society of Health-System Pharmacists*. 4th Edition, Bethesda, MD; 2009.*
- *Jorgensen J.H., Ferraro M.J. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices.* Clinical Infectious Diseases *2009;49:1749-1755.*

Interpreting Antibiotic Susceptibility Testing Results

One way to interpret the results of susceptibility tests is to determine the minimum inhibitory concentrations (MICs) for common pathogen-antibiotic (i.e., bug-drug) combinations. Once the MIC is determined for the antibiotic, breakpoints can be used to make a prediction of the likelihood of clinical success from the *in vitro* growth characteristics of the bug-drug combination.

Breakpoints are determined based on assimilation of moderate to large numbers of *in vitro* MICs for wild-type populations of bacteria (as opposed to including bacteria with acquired or selected resistance), assessment of how the antimicrobial behaves in an animal, how the antimicrobial inhibits the target organism at the site of infection, and studying the clinical outcome of infections when the antimicrobial is used.

Source:

- *Turnidge, John, and David Paterson. 2007. Setting and revising antibacterial susceptibility breakpoints.* Clinical Microbiology Reviews *20 (3): 391-408.*

Breakpoints are validated for a species (animal or human), a specific site of infection, a specific pathogen, and a specific drug and do not exist for all conditions in all animal species. Extrapolating susceptibility breakpoints from human data or from one animal species to another or to other disease conditions may lead to antibiotic treatment failure.

Reporting Susceptibility Test Results

Depending on the testing method, breakpoints are expressed as either a concentration (in milligrams per liter – mg/L or micrograms per milliliter – µg/ml) or a zone diameter (in millimeters – mm). In this example of *Mannheimia haemoltyica* from bovine lung, some antimicrobials and their breakpoints are provided. S, R, and I are defined as susceptible (S), intermediate (I), or resistant (R). As defined in the 2008 Clinical and Laboratory Standards Institute (CLSI) document M31-A3:

- "Susceptible: Implies that an infection due to the isolate tested may be appropriately treated with the dosage regimen of an antimicrobial agent recommended for that type of infection and animal species, unless otherwise indicated."
- "Intermediate: Implies an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated" (e.g., quinolones and β-lactams in urine) "or when a high dosage of drug can be used" (e.g., β-lactams);
  - This classification provides a 'buffer zone' due to the minor, random variations that can occur in antibiotic susceptibility testing where one time an antibiotic tests susceptible and the next time it tests resistant. CLSI states "a 'buffer zone' should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations."
- "Resistant: The isolate is not inhibited by the usually achievable concentrations of the agent with the normal dosage schedules and/or fall in the range where specific microbial resistance mechanisms are likely (e.g., β-lactamases), and clinical efficacy has not been reliable in treatment studies."

Bovine Lung: *Mannheimia haemolytica*

Antimicrobial	MIC µg/ml	
Ampicillin	< = 0.25	S
Ceftiofur	0.50	S
Clindamycin	8.0	R
Danofloxacin	<= 0.12	S
Florfenicol	0.50	S
Oxytetracycline	<= 0.50	S
Penicillin	<= 0.12	S
Spectinomycin	16.00	S
Sulfadimethoxine	> 256.00	R
Trimethoprim/Sulphamethoxazole	< = 2.00	I
Tulathromycin	2.00	S

Source:

- *CLSI. 2008. Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals; Approved Standard – Third Edition.* CLSI document M31-A3. *Wayne, PA:* Clinical and Laboratory Standards Institute*; 2008.*

Knowledge Review #2

In a disk diffusion susceptibility test, the disk with the largest inhibition zone diameter indicates that particular antibiotic that should be chosen for the best chance of success against that bacterium.

- ☐ True
- ☐ False

Answers are found in the appendix.

Pharmacokinetics and Pharmacodynamics

Understanding and interpreting pharmacokinetics (PK) and pharmacodynamics (PD) of a specific antibiotic is important when considering extra-label use (ELU) or if the pathogen is likely to have resistance mechanisms. Extra-label is the use of a drug in any manner other than what is on the label, which by definition means accompanying documents. Understanding the PK and PD of an antibiotic allows a veterinarian to construct the optimal treatment regimen for an animal – frequency, dose, duration, and route based on the expected microorganism and site of infection.

When using an antibiotic as described on the label for the species and indication with the appropriate assessment of susceptibility, it is not necessary to modify treatment regimens based on PK or PD. However, as described under breakpoints earlier, data is not available for every site of infection in every animal species; thus an understanding of PK/PD parameters is essential to a successful clinical outcome.

Source:

- *Rybak, M. 2006. Pharmacodynamics: Relation to antimicrobial resistance.* The American Journal of Medicine *119 (6 Suppl 1): S37-44.*

Pharmacokinetics

Pharmacokinetics (PK) describes the drug concentration in the body over time. This course is dependent on:

- Drug absorption: Movement of a drug into the bloodstream from the site of administration;
- Distribution: Movement of a drug to and from the blood and various tissues of the body;
- Metabolism: Chemical alteration of a drug by the body; and
- Excretion: Removal of a drug from the body.

Source:

- *Martinez, M.N. U.S. Food and Drug Administration. Applying Pharmacokinetics in Veterinary Pharmaceuticals Regulation. Accessed December 3, 2011 at: http://www.fda.gov/AnimalVeterinary/NewsEvents/FDAVeterinarian Newsletter/ucm088944.htm.*

Pharmacodynamics

Pharmacodynamics (PD) is the ability of an antibiotic to inhibit the target organism at the site of infection. It is determined by the interplay of the:

- Antibiotic's PK;
- Bacterial susceptibility;
- Antibiotic's mechanism of action; and
- Tissue microenvironment at the site of infection.

Classes of Antibiotic Agents based on PK/PD

Often pharmacokinetics and pharmacodynamics are combined to create the term PK/PD. When related to a specific antibiotic, the term represents the relationship between PK variables and bacterial inhibition or killing in the animal. By extension, PK/PD relates to clinical outcome. Based on PK/PD profiles of in vitro studies, antibiotic agents are commonly divided into two groups:

Classes of Antimicrobial Agents Based on PK/PD

PK/PD Profile	Antibiotic Agents	Goal of Therapy	PK/PD Parameter
1) Concentration Dependent	Fluoroquinolones Aminoglycosides Metronidazole	Maximize concentrations	AUC_{24}/MIC C_{max}/MIC
2) Time-Dependent w/Minimal or No Persistent Suppression of Bacterial Growth Following Exposure	Beta-lactams (including penicillins, cephalosporins, carbapenems, and monobactams)	Maximize duration of exposure	T>MIC

AUC = area under the curve; C_{max} = maximum concentration; T = time

With **concentration-dependent antibiotic agents** such as fluoroquinolones, killing becomes more rapid and profound with increasing drug concentrations. To maximize efficacy of dose-dependent antibiotics, increase the dose but keep dosing frequency the same.

With **time-dependent antibiotic agents** such as beta-lactams, it is the time above MIC that is important; increasing the drug concentration significantly does not necessarily increase efficacy. To maximize efficacy of time-dependent antibiotic agents, one may need to increase the dosing frequency.

For other antibiotic groups, the data supporting the use of a single parameter to optimize PK/PD relationships are not as clear as the data for beta-lactams, fluoroquinolones, and aminoglycosides. There are data that suggest that many of the antibiotics in these groups: Macrolides, tetracyclines, lincosamides, cyclic peptides, oxazolidinones, and potentiated-sulfas fall into the category of time-dependent, but further data will need to be gathered to confirm those assumptions.

Sources:

- *Owens, R., Shorr, A. 2009. Rational dosing of antimicrobial agents: Pharmacokinetic and pharmacodynamic strategies.* American Journal of Health-System Pharmacy *66 (12 Suppl 4): S23-30.*
- *Turnidge, J., Paterson, D. 2007. Setting and revising antibacterial susceptibility breakpoints.* Clinical Microbiology Reviews *20 (3): 391-408, table of contents.*

Knowledge Review #3

Based on PK/PD profiles, antibiotic agents are commonly divided into groups. Select ALL the correct statements below.

- **A.** Fluoroquinolones are concentration-dependent antibiotics
- **B.** Aminoglycosides are concentration-dependent antibiotics
- **C.** Macrolides are concentration-dependent antibiotics
- **D.** Beta-lactams are time-dependent antibiotics

Answers are found in the appendix.

Interpreting a Drug Label

Constructing a regimen based upon the PK/PD profile of an antibiotic provides patient- and pathogen-specific therapy and has the potential to make antibiotic therapy more effective. It is important to understand how to read a drug label in order to make the most informed drug choice. Below is an example of a concentration-dependent antibiotic demonstrating how important PK/PD parameters are illustrated in a drug label with definitions and interpretations following.

Pharmacokinetic Parameters Reflecting Total Drug Concentrations in Plasma (mean ± standard deviation) Following a 4.0mg/kg Subcutaneous Administration in Either the Middle Third or the Base of the Ear in Beef Cattle.

Pharmacokinetic Parameters	**MEAN ± SD[1]**	
	Beef – Middle Third of the Ear	**Beef –** Base of the Ear
Terminal plasma elimination half-life, $T_{1/2}$ (h)	68.4 ± 12.8	43.4 ± 11.5
AUC (µg-h/mL)	402 ± 78.4	435 ± 70.3
Time of maximum concentration, T_{max} (h)	13.0 ± 8.5	20.0 ± 9.3
Maximum concentration, C_{max} (µg/mL)	8.5 ± 3.01	8.9 ± 2.31
Vd_{ss} (L/kg)	0.125 ± 0.01	0.115 ± 0.02
CL_{total} (mL/h/kg)	0.83 ± 0.11	0.80 ± 0.13
[1]SD = standard deviation		

Description and interpretation of important PK/PD parameters:

- $T_{1/2}$
  - Apparent plasma elimination half-life ($T_{1/2}$) is the time required for the concentration in the plasma to decrease by half. $T_{1/2}$ is important as doubling the dose (in this example, 8.0 mg/kg) doubles the C_{max} (17.0 µg/ml) and adds one $T_{1/2}$ (68.4 h or 43.4 h depending on site of administration), but leaves the length of the $T_{1/2}$ unchanged.
- AUC
  - Area under plasma concentration curve (AUC) is one measure of drug exposure over time. For instance, AUC_{24} is drug absorption over a 24 hour period.
- T_{max}
  - The time it takes the drug to reach maximum plasma concentration. In this example, 13 or 20 hours depending on the site of administration.
- C_{max}
  - The maximum concentration (C_{max}) is the maximum drug concentration in plasma. In this example, 8.5 µg/ml or 8.9 µg/ml depending on the site of administration.
- Vd
  - The volume of distribution is one measure of the distribution of an antibiotic into the tissues.
- CL_{total}
  - The plasma clearance (CL_{total}) is the volume of plasma cleared of a drug per unit time (per hour in this example) and includes clearance by the liver and kidney; it is also often expressed in animals as per unit body weight.

PK/PD Parameters

Three PK/PD parameters have been demonstrated to predict the efficacy of antibiotics:

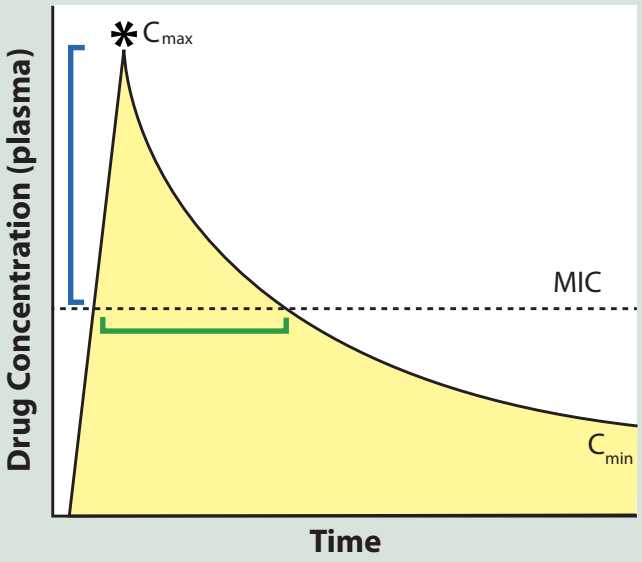
1. The ratio of the maximum serum concentration (C_{max}) to the minimum inhibitory concentration (MIC)
2. The ratio of the area under the plasma concentration time curve for a 24-hour interval (AUC_{24}) to the MIC
3. The percentage of time during a dosing interval that plasma concentrations exceed the MIC

Source:

- *Craig, W. A. 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men.* Clinical Infectious Diseases *26:1-12.*

The figure below presents these three PK/PD parameters in schematic form.

Pharmacodynamic parameters found to be important in describing the efficacy of different antibiotics.



AUC = area under the concentration-time curve

C_{min} = minimum (trough) plasma concentration

Concentration Dependent Antibiotics:

AUC_{24}:MIC = ratio of area under curve for 24hr interval above MIC

*C_{max}: MIC = max concentration of drug over MIC

Time Dependent Antibiotics:

Time>MIC = amount of time concentration is above MIC

Adapted from Rybak, MJ. Pharmacodynamics: Relation to Antimicrobial Resistance. *The American Journal of Medicine* (2006) Vol 119 (6A), S37-S44.

Likelihood of Success

AUC is clinically important as the likelihood of success when using concentration-dependent antibiotics (e.g., fluoroquinolones and aminoglycosides) is best predicted by AUC_{24}/MIC, with the most commonly reported values in the range of 100-125. For aminoglycosides, peak plasma concentration (C_{max}) should be 8-10 times the MIC value reported by the lab to maximize efficacy. In contrast, the likelihood of success when using time–dependent antibiotics (e.g., penicillin) depends on time above MIC. In general, exceeding MIC between 30% and 50% of the inter-dosing interval is appropriate for most time-dependent agents in non-debilitated patients.

Beyond understanding the PK/PD profile of the selected antibiotic, other determinants when selecting an antibiotic are also important to consider in veterinary medicine, including:

1. Compliance with dosing – route and frequency
2. Conservation of resources – animal handling, time, and supplies to treat
3. Cost-benefit ratio
4. Limiting toxicity of the drug in the patient (renal toxicity from aminoglycosides or retinal toxicity in cats from fluoroquinolones)

Source:

- *McKellar, Q.A., S.F. Sanchez Bruni, and D.G. Jones. 2004. Pharmacokinetic/pharmacodynamic relationships of antimicrobial drugs used in veterinary medicine.* Journal of Veterinary Pharmacology and Therapeutics *27 (6): 503-14.*

Example Antibiotic Calculation

You are treating a pyoderma in a canine patient. Based on the package insert (drug label) of your favorite fluoroquinolone (a concentration-dependent antibiotic), the average MIC for sensitive *Staphylococcus pseudintermedius* is listed as 0.25 µg/ml. The drug label also says that after an oral dose of 2.5mg/kg, the maximum tissue concentration in the skin is 0.66 µg/g of tissue. Your target concentration is 8-10x MIC.

0.25 µg/ml
x 8.00
2.00 µg/ml
target concentration

2.00 µg/ml
÷ 0.66 µg/g
≈ 3.00 times
label dose to reach target tissue concentrations

3.00 x 2.50 mg/kg = 7.50 mg/kg
target oral dose

Knowledge Review #4

Four important PK/PD parameters may be found on a drug label. Match the correct parameter with the correct description.

A.	AUC	**1.**	Maximum plasma drug concentration
B.	CL_{total}	**2.**	An indication of the extent of drug absorption
C.	C_{max}	**3.**	Indicates how fast the drug is cleared from plasma
D.	$T_{1/2}$	**4.**	Time required for the concentration in the plasma to decrease by half

Answers are found in the appendix.

Antibiotic Regulation – Federal and State

There are both federal and state level regulations of antibiotics and their use in animals.

Federal regulation of antibiotics occurs through the U.S. Food and Drug Administration's Center for Veterinary Medicine (CVM). The CVM approves all antibiotics used in animals. The CVM approves antibiotics for four uses and as one of three marketing types:

Uses:

1. Disease Treatment: Drug administered only to animals exhibiting clinical signs of disease;
2. Disease Control: Drug administered to a group of animals when a proportion of the animals in the group exhibit clinical signs of disease;
3. Disease Prevention: Drug administered to a group of animals, none of which are exhibiting clinical signs of disease, in a situation where disease is likely to occur if the drug is not administered; and
4. Production or Growth-enhancing purposes*: Drug administered, typically through feed or water, to growing, healthy animals to promote increased gain in body weight over a defined period of time, or improved conversion of feed to body mass.

1. **Disease Treatment**
2. **Disease Control**
3. **Disease Prevention**
4. **Production or Growth-enhancing**

*FDA proposed changes to this use in April 2012. See below for further explanation.

Types (more information on each of these is presented later):

1. Over-the-counter (OTC)
2. Veterinary prescription (Rx)
3. Veterinary feed directive (VFD)**

1. **Over-the-Counter (OTC)**
2. **Veterinary Prescription (RX)**
3. **Veterinary Feed Directive (VFD)**

**FDA proposed changes to this type in April 2012. See below for further explanation.

Source:

- *William Flynn, DVM, FDA Center for Veterinary Medicine, personal communication, October 13, 2011*

FDA Proposed Changes, April 2012

FDA Guidance for Industry (GFI) #209, The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals, published April 13, 2012 discusses efforts to use medically important antimicrobials as judiciously as possible to minimize antimicrobial resistance development and preserve effectiveness in humans and animals. FDA has proposed two additional principles to address this issue: 1) The use of medically important antimicrobial drugs in food-producing animals should be limited to those uses that are considered necessary for assuring animal health. FDA considers the use of medically important antimicrobials in feed and water for production purposes an injudicious use; and 2) The use of medically important antimicrobial drugs in food-producing animals should be limited to those uses that include veterinary oversight or consultation. GFI #209 is available at: *http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf*

FDA is suggesting ***voluntary*** compliance from drug sponsors to move from OTC use in feed to a VFD and OTC use in water to Rx. The full details are described in GFI#213, New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209. GFI #213 is available at: *http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM299624.pdf*

State regulation of the use of antibiotics in animals occurs through the State Board of Veterinary Medicine. In addition, state pharmacy acts describe legal prescriptions and distribution of drugs. Veterinarians are required to be familiar with and abide by the laws and regulations within the state(s) in which they are licensed.

Veterinarian-Client-Patient Relationship (VCPR)

The Veterinarian-Client-Patient-Relationship (VCPR) is the basis for interaction among veterinarians, their clients, and their patients. Without a VCPR, it is unethical and illegal under most state laws to merchandise or use (on-label or extra-label) **prescription drugs**. In addition, a federally defined VCPR is always required when using an approved animal or human drug in an **extra-label manner** in animals.

As defined in 21CFR 530.3, a valid veterinarian-client-patient-relationship (VCPR) for extra-label drug use is one in which:

- A veterinarian has assumed the responsibility for making medical judgments regarding the health of (an) animal(s) and the need for medical treatment, and the client (the owner of the animal or animals or other caretaker) has agreed to follow the instructions of the veterinarian;
- There is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s); and
- The practicing veterinarian is readily available for follow-up in case of adverse reactions or failure of the regimen of therapy. Such a relationship can exist only when the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of examination of the animal(s), and/or by medically appropriate and timely visits to the premises where the animal(s) are kept.

Sources:

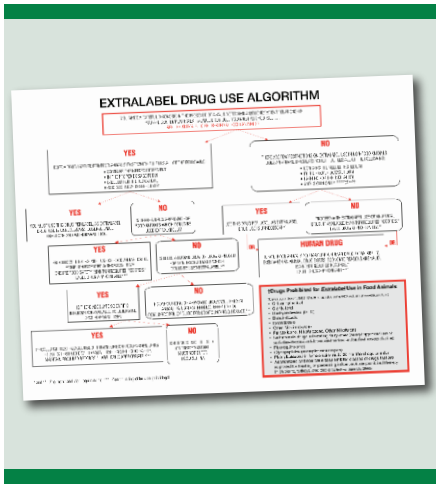
- *Code of Federal Regulations, Title 21, Section 530.3 accessed January 4, 2012 at: http://www.access.gpo.gov/nara/cfr/waisidx_04/21cfr530_04.html*
- *VCPR Requirement for Prescriptions,* American Veterinary Medical Association*, updated January 2012, accessed May 17, 2012 at: http://www.avma.org/advocacy/state/issues/VCPR_and_prescriptions.asp.*

General Conditions for Extra-Label Use of Drugs Under AMDUCA

Passed in 1994, the Animal Medicinal Drug Use Clarification Act (AMDUCA) allows veterinarians to prescribe certain approved drugs for extra-label use (ELU) under defined conditions.

Situations in which extra-label use of drugs may be considered include when:

- There is no animal drug approved for the intended use;
- There is an animal drug approved for the intended use, but the approved drug is not in the required dosage form or concentration;
- The approved drug has been found to be clinically ineffective when used as labeled; or
- The intended use is in a non-food animal, then an approved human drug can be used even if an approved animal drug is available.

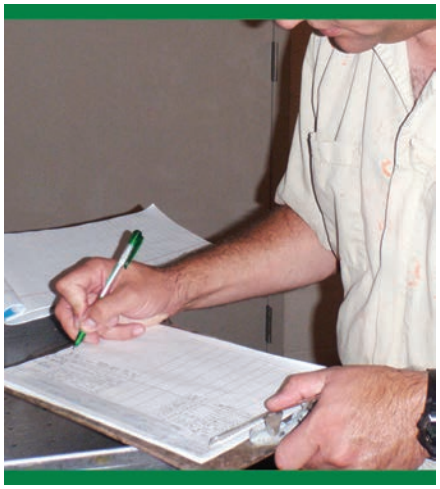


ELU of a drug is permitted only by or on the order of a licensed veterinarian with a federally-defined valid VCPR in place. ELU is allowed only for FDA approved animal and human drugs and is intended for therapeutic use only, not production purposes. ELU of an approved drug must not result in violative drug residues in food posing a risk to public health. AMDUCA does NOT permit ELU of medicated animal feed under any circumstances. All ELU must be in conformance with 21CFR530.

Source:

- *Code of Federal Regulations, Title 21, Section 530, accessed January 4, 2012 at: http://www.access.gpo.gov/nara/cfr/waisidx_10/21cfr530_10.html*

The AVMA created an Extra-Label Drug Use Algorithm (2007) accessible at: *http://www.avma.org/reference/amduca/extralabel_brochure.pdf*



ELU Recordkeeping Requirements

The FDA also has specific ELU recordkeeping requirements. The veterinarian must maintain records that include:

- The established name of the drug and its active ingredient, or if formulated from more than one ingredient, established name of each ingredient;
- Condition treated;

- Species of the treated animal(s);
- Dosage administered;
- Route of administration;
- Treatment duration;
- Number of animals treated;
- Animal's identification
  - In food animal practices, identification can be on a group, herd, flock, or per-client basis; and
- Withdrawal, withholding, or discard time(s) for meat, milk, eggs, or any food product from the animal(s) treated.

The veterinarian must keep these records for **at least two years** as required by Federal law. State recordkeeping requirements may be longer. These records must be available at any reasonable times to FDA designated personnel for copying and verifying.

ELU Labeling Requirements

There are also labeling requirements for extra-label use of drugs. The label must have the following information:

- Name and address of the prescribing veterinarian (and the pharmacy if dispensed this way);
- Established name of the drug, or if formulated from more than one active ingredient, the established name of each ingredient;
- Directions for use including the class/species or identification of the animal or herd, flock, pen, lot, or other group of animals being treated, in which the drug is intended to be used; the dosage, frequency, and route of administration, and the duration of therapy;
- Animal identification (individual for companion animals, or group/pen for food animals if prescribed this way);
- Withdrawal intervals; and
- Any cautionary statements (e.g., do not use in horses intended for human consumption).

Use of Approved Human Drugs in Food-Producing Animals

When considering ELU of an approved human drug in food-producing animals, the prescribing veterinarian:

- Must have a therapeutic rationale for the use;
- May not use an approved human drug in an ELU manner if an animal drug approved for use in a food-producing animal species can be used; and
- Must take appropriate measures to assure that the animal and its food products will not enter the human food supply if scientific information on the human food safety aspect of the use of the drug in food-producing animals is not available.

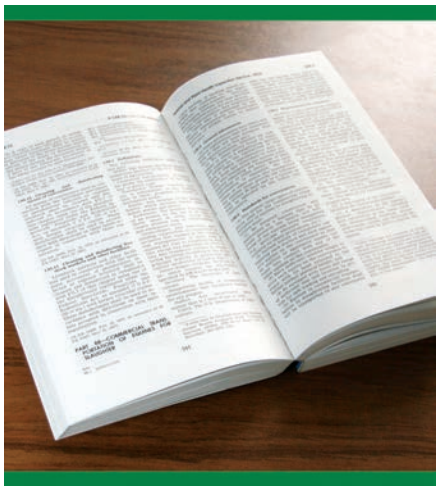
Before prescribing or dispensing an approved animal or human drug for extra-label use in food-producing animals, the veterinarian must:

- Make a careful diagnosis and evaluation of the conditions for which the drug is to be used;
- Provide an estimated, scientifically-based, withdrawal interval for the milk, meat, eggs, or other edible products from the treated animal (this information may be obtained by the veterinarian in context of a VCPR from among other sources, scientific literature, academia, or the Food Animal Residue Avoidance Databank (FARAD)*;
- Make sure that the identity of the treated animal or animals is maintained; and
- Take measures to assure that assigned timeframes for withdrawal are met and no illegal drug residues occur in any food-producing animal subjected to extra-label treatment.

*Food Animal Residue Avoidance Databank (FARAD) is a national, USDA-sponsored, cooperative project, with a primary mission to prevent or mitigate illegal residues of drugs, pesticides and other chemicals in foods of animal

origin. Their website, *www.farad.org*, contains FARAD Digests to look up withdrawal recommendations; users can also submit a question or receive advice regarding drug withdrawal intervals.

Drugs Prohibited by FDA for ELU in Food-Producing Animals



The FDA also keeps a list of drugs, not limited to antibiotics, prohibited from extra-label use in food-producing animals (21CFR530.41). As of April 5, 2012, this list includes:

1. Chloramphenicol
2. Clenbuterol
3. Diethylstilbestrol (DES)
4. Dimetridazole
5. Ipronidazole
6. Other nitroimdazoles
7. Furazolidone
8. Nitrofurazone
9. Sulfonamide drugs in lactating dairy cattle
10. Fluoroquinolones
11. Glycopepetides
12. Phenylbutazone in female dairy cattle 20 months of age or older.
13. Cephalosporins (not including cephapirin) in cattle, swine, chickens, or turkeys:
    i. For disease prevention purposes;
    ii. At unapproved doses, frequencies, durations, or routes of administration; or
    iii. If the drug is not approved for that species and production class.
14. The following drugs, or classes of drugs, that are approved for treating or preventing influenza A, are prohibited from extralabel use in chickens, turkeys, and ducks: Adamantanes and neuraminidase inhibitors.

Sources:

- *Code of Federal Regulations Title 21, Part 530.41 Drugs prohibited for extralabel use in animals. Accessed November 15, 2011 at: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=530.41*
- *Federal Register Volume 77, Number 4, January 6, 2012, pages 735-745 accessed April 16, 2012 at: http://www.gpo.gov/fdsys/pkg/FR-2012-01-06/html/2012-35.htm*

Knowledge Review #5

A valid VCPR for extra-label use of a drug includes all of the following EXCEPT:

A. A veterinarian has assumed the responsibility for making medical judgments regarding the health of (an) animal(s) and the need for medical treatment
B. The client has agreed to follow the instructions of the veterinarian
C. There is a confirmed diagnosis
D. The practicing veterinarian is readily available for follow-up
E. The veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s)

Answers are found in the appendix.

Feed Antimicrobials

The FDA approves animal drugs used in or on animal feed for therapeutic uses (i.e., to prevent, control, or treat certain diseases) or for production uses (i.e., promoting growth or increasing feed efficiency).

Animal drugs dispensed in or on animal feed that require professional veterinary supervision are regulated by the FDA under the Veterinary Feed Directive (VFD). The VFD was established by Congress as part of the Animal Drug Availability Act of 1996 (ADAA). Prior to ADAA, all medicated animal feed was dispensed over-the-counter (OTC). The ability to write a veterinary feed directive provides veterinarians a prescription-like status for certain animal drugs when used in or on animal feed.

"FDA regulations in Title 21 Code of Federal Regulations (CFR), Part 558.3(b)(7) defines 'veterinary feed directive' as a written statement issued by a licensed veterinarian in the course of the veterinarian's professional practice that orders the use of a VFD drug in or on an animal feed. This written statement authorizes the client to obtain and use the VFD drug in or on animal feed to treat the client's animals only in accordance with the directions for use approved or indexed by FDA. A veterinary feed directive is also referred to as a VFD order."

Source:

- *FDA CVM Guidance for Industry 120, Veterinary Feed Directive Regulation Questions and Answers, Final Guidance, March 26, 2009 accessed at: http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052660.pdf*

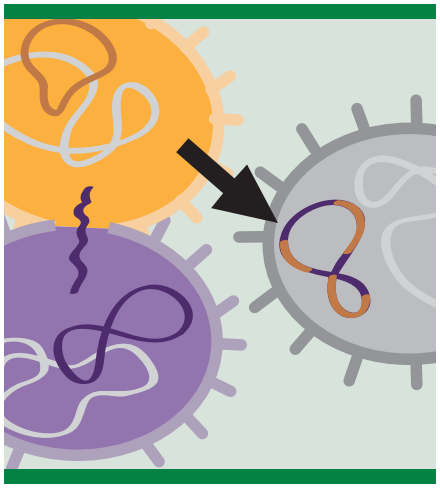
When a new animal drug application is submitted to the FDA's CVM for approval, the appropriate review division determines whether the drug will be an OTC drug, a prescription drug (for non-feed use), or a VFD drug (for animal feed use). The list of VFD drugs changes over time with these new approvals. For current information on the VFD, please visit the FDA website at: *http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm071807.htm*

For more information on the Veterinary Feed Directive (VFD), please visit:

- FDA Guidance for Industry #120: Veterinary Feed Directive Regulation Questions and Answers, Final Guidance, March 2009 *http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052660.pdf*
- U.S. Code Title 21, Chapter 9, subchapter V, Part A, 354 Veterinary feed directive drugs (Feb 2010): *http://frwebgate.access.gpo.gov/cgi-bin/usc.cgi?ACTION=RETRIEVE&FILE=$$xa$$busc21.wais&start=918350&SIZE=5135&TYPE=PDF*
- Title 21 Code of Federal Regulations, Part 558, Veterinary Feed Directive; Draft Text for Proposed Regulation, April 13, 2012: *http://www.gpo.gov/fdsys/pkg/FR-2012-04-13/pdf/2012-8844.pdf*

Antimicrobial Resistance

Microbes (bacteria, viruses, fungi or parasites) are living organisms that adapt to their environment and change to ensure their survival. Antimicrobial resistance (AMR) is the ability of a microbe to grow in the presence of a chemical that would normally kill or limit the microbe's growth. Resistant organisms are able to survive despite the presence of antimicrobials, such as antibiotics, antivirals, antifungals, and antimalarials. Some antimicrobials are losing their effectiveness after more than 50 years of widespread use.



Microbes change their genetic makeup allowing survivors to pass on these new genes to their offspring and, in some cases, to neighboring microbes. The survivors' progeny will quickly become the dominant type throughout the microbial population. This ensures that the population of resistant microbes persists making it harder to eliminate infections.

The development of antimicrobial resistance is not new or unexpected. Once antimicrobials are introduced, resistance will emerge as evidenced since the first use in humans and animals. Both natural causes and societal pressures drive microbes to constantly adapt in an effort to evade the antimicrobials developed to kill them.

Sources:

- *Besser, T. Mechanisms of Antimicrobial Resistance, American Association of Bovine Practitioners Annual Meeting, 2011.*
- *Flynn, W. FDA Activities and AMR Policies, American Association of Bovine Practitioners Annual Meeting, 2011.*

Describing the mechanisms of AMR is beyond the scope of this module, so reputable resources are provided below to learn more. In a general sense, AMR can occur intrinsically* or be acquired**. Inappropriate use of medicines, including poor antibiotic selection, dose, treatment duration, route of administration, and compliance in animals and humans, provides favorable conditions for resistant microorganisms to emerge and spread.

*Intrinsic antimicrobial resistance occurs when bacteria naturally have structural or functional characteristics that resist activity to a particular antibiotic agent. This can occur if the organism lacks target binding sites or structures or produces an enzyme that inactivates the drug. For example, *Mycoplasma* lack a cell wall and therefore antibiotics that target the cell wall such as penicillins and cephalosporins are not effective against mycoplasma. Source: Intrinsic Resistance, Antimicrobial Resistance Learning Site for Veterinary Students. Michigan State University. Accessed May 21, 2012 at: *http://amrls.cvm.msu.edu/microbiology/molecular-basis-for-antimicrobial-resistance/intrinsic-resistance*

**Acquired resistance occurs when a particular bacterium (not all strains or subpopulations) undergoes spontaneous mutation of a gene or horizontal gene transfer (which occurs through transformation, transduction or conjugation). Source: Acquired Resistance, Antimicrobial Resistance Learning Site for Veterinary Students. Michigan State University. Accessed May 21, 2012 at: *http://amrls.cvm.msu.edu/microbiology/molecular-basis-for-antimicrobial-resistance/acquired-resistance*

Judicious use of antibiotics can help combat the development of AMR. Judicious use targets the primary pathogen, recognizing that other pathogens are also exposed to the antibiotic. Judicious use also means knowing or being able to predict the MIC of the target pathogen and selecting the appropriate antibiotic, dose, treatment duration, route, frequency, and route.

For more information on AMR, see:

- World Health Organization, Antimicrobial Resistance Fact Sheet 194, February 2011, accessed December 15, 2011 at: *http://www.who.int/mediacentre/factsheets/fs194/en/http://www.who.int/mediacentre/factsheets/fs194/en/*
- Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Antimicrobial (Drug) Resistance – History and A Growing Health Issue. Last updated January 12, 2012. Accessed January 20, 2012 at: *http://www.niaid.nih.gov/topics/antimicrobialResistance/Understanding/Pages/default.aspx*
- Tenover, F.C. Mechanisms of Antimicrobial Resistance in Bacteria. *American Journal of Medicine*. Vol. 119 (6A), June 2006.

Monitoring Antibiotic Residues and Resistance

Monitoring antibiotic residues* in meat and animal products is crucial to maintaining a safe food supply and protecting public health. Likewise, antimicrobial resistance monitoring has increased in importance as human drugs have lost their efficacy over time. There are various federal agencies responsible for monitoring these two different sequelae of antibiotic use in animals.

*A residue is a chemical compound found in food animal and egg products, including approved (legal) and unapproved (illegal) veterinary drugs, pesticides, hormones, and environmental products. Source: U.S. National Residue Program Scheduled Sampling Plans ("Blue Book"), 2011: *http://www.fsis.usda.gov/PDF/2011_Blue_Book.pdf*

Federal Agencies Monitoring Residues

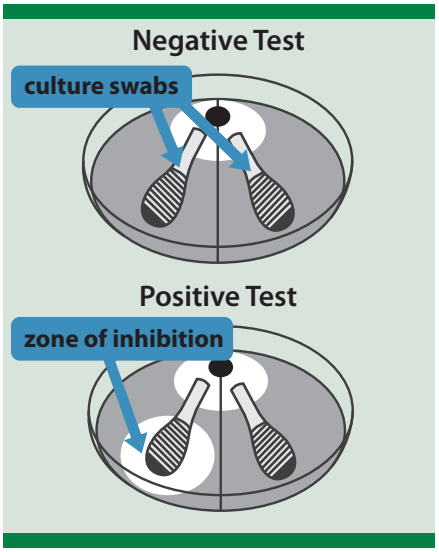
The United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) protects consumers by ensuring that USDA inspected meat, poultry, and egg products are safe, wholesome, accurately labeled, and do not contain illegal drug residues. FSIS works with the Environmental Protection Agency (EPA) and FDA to achieve the goals of the National Residue Program (NRP).

- FDA establishes the veterinary drug tolerance levels in food products (21CFR)
- EPA establishes pesticide tolerances (40CFR)
- FSIS enforces the tolerance levels

The National Residue Program provides a yearly scheduled sampling plan for testing chemical compounds in products from food animals and egg products produced domestically or imported into the United States. The United States NRP Blue Book provides a summary of the sampling plans and can be accessed at: *http://www.fsis.usda.gov/Science/2011_Blue_Book/index.asp*.

Domestic sampling for residues in meat occurs in one of two ways:

1. Scheduled random sampling of tissues taken from food animals that have passed antemortem inspection; and
2. Inspector-generated sampling where Public Health Veterinarians conduct sampling in-plant on animals suspected of having violative levels of chemical residues. The inspector will select a carcass for sampling based on professional judgment and public health criteria outlined in FSIS Directives 10,800.1 and 10,220.3, including:
   - Animal disease signs;
   - Producer history; or
   - Results from random-scheduled sampling.



RESIDUE DATABASE

The Residue Violation Information System (RVIS) is a national interagency (FSIS, FDA, EPA and States) database with information on residue violations in livestock and poultry slaughtered in the U.S. It is published weekly and includes all types of residues (drugs, pesticides, heavy metals). To access the database, visit: *http://www.fsis.usda.gov/science/Chemistry/index.asp#nrp*

RESIDUE AVOIDANCE

A resource for veterinarians faced with making ELU decisions for their patients is the Food Animal Residue Avoidance Databank (FARAD). This national, USDA-sponsored, cooperative project has the primary mission to prevent or mitigate illegal residues of drugs, pesticides, and other chemicals in foods of animal origin. On the FARAD website, individuals may look up withdrawal interval recommendations of drugs published in the FARAD digests. If a drug of interest is not listed, a question or request for advice can be submitted via online forms. For more information, please visit the FARAD website at: *www.farad.org*.

Federal Agencies Monitoring Resistance

The National Antimicrobial Resistance Monitoring System (NARMS) is a cooperative program among the FDA, the Centers for Disease Control and Prevention (CDC) and USDA. NARMS monitors antimicrobial susceptibility among enteric bacteria from humans, retail meats, and food animals at slaughter. Each NARMS agency publishes comprehensive annual reports and an annual Executive Report integrates the isolate data and is published on the FDA website. The program also collaborates with similar systems in other countries, working towards international coordination of testing and reporting. For more information please visit: *http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm*

Knowledge Review #6

Match the following agencies and programs with their correct role in antibiotic regulation or antimicrobial resistance monitoring.

A.	FSIS	**1.**	Program that monitors antimicrobial susceptibility among enteric bacteria from humans, retail meats, and food animals
B.	FDA	**2.**	Establishes the veterinary drug tolerance levels in food products
C.	EPA	**3.**	Establishes pesticide tolerances
D.	FARAD	**4.**	Cooperative project that prevents or mitigates illegal residues of drugs, pesticides, and other chemicals in foods of animal origin
E.	RVIS	**5.**	Ensures that USDA inspected meat, poultry, and egg products are safe and do not contain illegal residues
F.	NARMS	**6.**	National interagency database with information on residue violations in livestock and poultry slaughtered in the U.S.

Answers are found in the appendix.

Staying Current with Antibiotic Regulations and Issues

The following is a list of website resources that you can visit to learn more about antibiotic drug approval and use in animals and how it relates to human health concerns and safety.

- Antimicrobial Resistance Learning Site, Michigan State University, University of Minnesota, and CDC *http://amrls.cvm.msu.edu/*
  - Online, interactive suite of educational materials aimed at teaching and promoting the prudent use of antibiotics in animal agriculture and veterinary medicine

- AVMA Scientific Reference and Government Materials *https://ebusiness.avma.org/EBusiness50/ProductCatalog/ProductCategory.aspx?ID=138*
  - A variety of downloadable documents on judicious use of antimicrobials, extra-label drug use, pharmaceutical disposal, compounding, biologics, and more

- Centers for Disease Control and Prevention (CDC) *http://www.cdc.gov/drugresistance/index.html*
  - FAQs, diseases/pathogens associated with antimicrobial resistance, resources, laboratory testing and training resources, education and campaigns, surveillance systems

- Code of Federal Regulations, Title 21, Chapter 1, Part 530 Extralabel drug use in animals *http://www.access.gpo.gov/nara/cfr/waisidx_10/21cfr530_10.html*

- Food Animal Residue Avoidance Databank (FARAD) *www.farad.org/*
  - FARAD is a national, USDA-sponsored, cooperative project, with a primary mission to prevent or mitigate illegal residues of drugs, pesticides and other chemicals in foods of animal origin.
  - Submit a question or receive advice regarding drug withdrawal intervals
  - Look up withdrawal interval recommendations published in the FARAD Digests

- FDA Green Book – Approved Animal Drug Products *http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/ucm042847.htm*
  - A resource, updated monthly, of FDA approved animal drug products including active ingredients of various drugs, patent information and drugs withdrawn from the market.

- FDA Guidance for Industry #120: Veterinary Feed Directive Regulation Questions and Answers, Final Guidance, March 2009 *http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052660.pdf*

- FDA Guidance for Industry #209: The Judicious Use of Medically Important Antimicrobial Drugs in Food-Producing Animals. *http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM216936.pdf*

- FDA Guidance for Industry #213: New Animal Drugs and New Animal Drug Combination Products Administered in or on Medicated Feed or Drinking Water of Food-Producing Animals: Recommendations for Drug Sponsors for Voluntarily Aligning Product Use Conditions with GFI #209 Draft Guidance *http://www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM299624.pdf*

- Legislative Issues on Antimicrobial Drugs (followed by AVMA) *http://www.avma.org/advocacy/federal/regulatory/public_health/judicious_use_antimicrobial_drugs.asp*
  - The AVMA Governmental Relations Division provides details about the various bills being considered in Congress.

- National Antimicrobial Resistance Monitoring System (NARMS) is available on the FDA website: *http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/default.htm*
  USDA website: *http://www.ars.usda.gov/Main/docs.htm?docid=6750*
  CDC website: *www.cdc.gov/narms*
  - NARMS is a multi-faceted monitoring system that provides data to regulatory officials and the veterinary medical community to help assess the risk associated with antimicrobial use in food animal production

- National Milk Producers Federation *http://www.nationaldairyfarm.com/residue-prevention.html*
  - Featuring the "Milk and Dairy Beef Drug Residue Prevention Manual" with information concerning drug residues, approved drugs, and record keeping forms

- U.S. Code Title 21, Food and Drugs, Chapter 9 Federal Food, Drug and Cosmetic Act, Subchapter V, Drugs and Devices *http://frwebgate.access.gpo.gov/cgi-bin/usc.cgi?ACTION=BROWSE&TITLE=21USCC9&PDFS=YES*

Summary

This module introduced the terms and concepts necessary for veterinarians to make informed decisions for the proper selection and judicious use of antibiotics in animals. The various benefits and limitations of antibiotic susceptibility testing options as well as correctly interpreting a drug label were presented. There are many agencies involved in antibiotic regulation and antibiotic resistance and residue monitoring. A review of the key components of the Animal Medicinal Drug Use Clarification Act (AMDUCA) provided information about extra-label use of drugs. As new information on use and resistance emerges, utilize the resources presented here to assist in making informed decisions on antibiotic use in animals.

Resources/Web Links

Throughout this module, you were provided with multiple links to information and resources. Many of these links are repeated here.

- Beef Quality Assurance National Manual
  http://www.bqa.org/CMDocs/bqa/NationalManual.pdf

- Dairy Animal Care and Quality Assurance Manual
  http://www.bqa.org/CMDocs/bqa/DairyBQAManual.pdf

- Pork Quality Assurance guidance
  http://www.pork.org/filelibrary/PQAPlus/PQAPlusEdBook.pdf

- FDA Veterinary Feed Directive
  http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/ucm071807.htm

- U.S. Code, Title 21, Chapter 9, subchapter V, Part A, 354 Veterinary feed directive drugs, Feb 2010
  http://frwebgate.access.gpo.gov/cgi-bin/usc.cgi?ACTION=RETRIEVE&FILE=$$xa$$busc21.wais&start=918350&SIZE=5135&TYPE=PDF

- U.S. Code of Federal Regulations, Title 21, Part 558, Veterinary Feed Directive; Draft Text for Proposed Regulation, April 13, 2012
  http://www.gpo.gov/fdsys/pkg/FR-2012-04-13/pdf/2012-8844.pdf

- World Health Organization, Antimicrobial Resistance Fact Sheet 194, February 2011
  http://www.who.int/mediacentre/factsheets/fs194/en/http://www.who.int/mediacentre/factsheets/fs194/en/

- Department of Health and Human Services, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Antimicrobial (Drug) Resistance – History and A Growing Health Issue, January 2012
  http://www.niaid.nih.gov/topics/antimicrobialResistance/Understanding/Pages/default.aspx

- The United States National Residue Program (NRP) Blue Book
  http://www.fsis.usda.gov/Science/2011_Blue_Book/index.asp

- Residue Violation Information System
  http://www.fsis.usda.gov/science/Chemistry/index.asp#nrp

Acknowledgements

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The content of this module has been reviewed and approved by USDA-APHIS Legislative and Public Affairs.

Photo and Illustation Credits

Page 1 This is a photo of multiple vials of penicillin, a common antibiotic, in the refrigerator. *Graphic illustration by: Dani Ausen, Iowa State University*

Page 2 ***(Top)*** This is a photo of culture media, specifically agar plates. *Photo source: Andrew Kingsbury, Iowa State University*
(Center) A technician performs a Gram stain. *Photo source: Andrew Kingsbury, Iowa State University*
(Bottom) This is a graphic depiction of two antibiotic resistance mechanisms, efflux and restricted uptake. *Graphic illustration by: Dani Ausen, Iowa State University*

Page 3 This dog has a high concentration of penicillin in his urine. *Photo source: Dani Ausen, Iowa State University*

Page 4 ***(Top)*** Withdrawal times for antibiotics used in food-producing animals must be strictly adhered to. *Graphic illustration by: Dani Ausen, Iowa State University*
(Bottom) This photo shows a pig receiving an injection in the neck, an appropriate route of intramuscular antibiotic administration in this species. *Photo source: Alex Ramirez, Veterinary Diagnostic and Production Animal Medicine, Iowa State University*

Page 5 ***(Top)*** This photo depicts a blood agar plate being streaked. *Photo source: Andrew Kingsbury, Iowa State University*
(Bottom) This graphic depicts the first three steps involved in the disk diffusion method of antibiotic susceptibility testing: streaking an agar plate (1), dispensing antibiotic disks (2), and incubating the agar plate (3). *Photo source: Andrew Kingsbury, Iowa State University (all); Graphic illustration by: Dani Ausen, Iowa State University*

Page 6 This graphic depicts the final three steps involved in the disk diffusion method of observing bacterial growth (4), measuring the IZD (5), and consulting a standard interpretation chart (6). *Photo source: Andrew Kingsbury, Iowa State University (all); Graphic illustration by:Dani Ausen, Iowa State University*

Page 7 ***(Top)*** This graphic depicts steps one and two involved in the broth microdilution method of antibiotic susceptibility testing: using a sterile plate with different concentrations of antibiotic agents (1), and inoculating each well with bacterial isolate (2). *Photo source: Andrew Kingsbury, Iowa State University (all); Graphic illustration by: Dani Ausen, Iowa State University*
(Center) This graphic depicts steps three, four, and five involved in the broth microdilution method of antibiotic susceptibility testing: incubating the microtiter plate (3), visually evaluating each well for growth (4), and evaluating each well for growth using a computer (5). *Photo source: Andrew Kingsbury, Iowa State University (all); Graphic illustration by: Dani Ausen, Iowa State University*
(Bottom) This graphic depicts steps six and seven involved in the broth microdilution method of antibiotic susceptibility testing: qualifying each MIC value (6), andanalyzing the MIC values for different antibiotics tested (7). *Photo source: Andrew Kingsbury, Iowa State University (both); Graphic illustration by: Dani Ausen, Iowa State University*

Page 8 This graphic depicts steps six and seven involved in the broth microdilution method of antibiotic susceptibility testing: analyzing the MIC values for different antibiotics tested (8) and reporting the results (9). *Photo source: Andrew Kingsbury, Iowa State University (both); Graphic illustration by: Dani Ausen, Iowa State University*

Page 9 ***(Top)*** This graphic depicts the first three steps involved in the antibiotic concentration gradient method of antibiotic susceptibility testing: streaking the solid media agar (1), placing antibiotic impregnated Etest strips on the agar (2), and incubating the agar plate (3). *Photo source: Andrew Kingsbury, Iowa State University (all); Graphic illustration by: Dani Ausen, Iowa State University*
(Bottom) This graphic depicts steps four and five involved in the antibiotic concentration gradient method of antibiotic susceptibility testing: observing bacterial growth (4), and interpreting the results (5). *Photo source: Andrew Kingsbury, Iowa State University (both); Graphic illustration by: Dani Ausen, Iowa State University*

Page 10 This graphic depicts the final step involved in the antibiotic concentration gradient method of antibiotic susceptibility testing: reporting the results (6). *Photo source: Andrew Kingsbury, Iowa State University; Graphic illustration by: Dani Ausen, Iowa State University*

Page 11 This graphic depicts the minimum inhibitory concentrations (MICs) of various antimicrobials against *Mannheimia haemolytica* in the bovine lung. *Graphic illustration by: Andrew Kingsbury, Iowa State University*

Page 12 The two classes of antimicrobial agents based on their PK/PD profile include concentration dependent and time dependent. Example antibiotic agents, therapy goals and PK/PD paramters are listed in the table. *Graphic illustration by: Kate Harvey, Iowa State University*

Page 13 This chart depicts the various pharmacokinetic parameters that must be understood to make the most informed drug choice. *Graphic illustration by: Kate Harvey, Iowa State University*

Page 14 This graph depicts pharmacodynamic parameters found to be important in describing the efficacy of different antibiotics. *Graphic illustration by: Kate Harvey, Iowa State University*

Page 15 This graphic works through the math needed to determine a target oral dose. *Graphic illustration by: Andrew Kingsbury, Iowa State University*

Page 16 ***(Top)*** This graphic lists the four FDA CVM approved uses for antibiotics. *Graphic illustration by: Dani Ausen, Iowa State University*

(Bottom) This graphic lists the three FDA CVM approved marketing types for antibiotics. *Graphic illustration by: Dani Ausen, Iowa State University*

Page 17 ***(Top)*** This photo shows a veterinarian talking with a producer on-site. *Photo source: Bryan Buss, Iowa State University*

(Center) This graphic is a thumbnail of the Extra-Label Drug Use Algorithm Brochure put together by AVMA. *Graphic illustration by: Dani Ausen, Iowa State University*

(Bottom) Veterinarians must be sure to follow all FDA ELU recordkeeping requirements. *Photo source: Alex Ramirez, Veterinary Diagnostic and Production Animal Medicine, Iowa State University*

Page 18 ***(Top)*** This label includes all the details required for an extra-label use of a drug. *Graphic illustration by: Dani Ausen, Iowa State University*

(Bottom) Extra-label use of drugs in food-producing animals like this sheep need to have appropriate meat withdrawal times on the antibiotic label, along with the other details previously discussed. *Photo source: Danelle Bickett-Weddle, Iowa State University; Graphic illustration by: Andrew Kingsbury, Iowa State University*

Page 19 CFR Page 21CFR530.41 contains a list of drugs prohibited from extra-label use in food-producing animals. *Photo source: Andrew Kingsbury, Iowa State University*

Page 20 ***(Top)*** This photo shows young turkeys eating at a feeder. Certain animal drugs may be used in or on animal feed for therapeutic or production uses. *Photo source: Lara Durben, Minnesota Turkey Growers Association*

(Bottom) Graphic depiction of microbes exchanging nucleic acids and their offspring has these new genes making them resistant. *Graphic illustration by: Dani Ausen, Iowa State University*

Page 21 Various federal agencies monitor antibiotic residues and resistance in meat and animal products to keep the food supply safe. *Photo source: Danelle Bickett-Weddle, Iowa State University*

Page 22 ***(Top)*** FSIS Public Health Veterinarians may sample carcasses for antibiotic residues using culture swabs. The swabs are then placed on an agar plate that has been streaked with a harmless bacterium and then incubated overnight. A plate without a zone of inhibition indicates a negative test for an antibiotic residue (top image). A zone of inhibition around the swab indicates a positive test (bottom image), i.e., the carcass has an antibiotic residue. *Graphic illustration by: Dani Ausen, Iowa State University*

(Bottom) The FARAD website is pictured. *Graphic illustration by: Dani Ausen, Iowa State University*

Page 24 This photo depicts a microtiter plate sitting next to bottle of injectable antibiotics. *Photo source: Andrew Kingsbury, Iowa State University*

Knowledge Review Answers

Knowledge Review #1

Various parameters should be considered to assure proper antibiotic selection and optimize success. Select ALL that apply.

- **A.** Site of infection
- **B.** Animal species
- **C.** Culture results
- **D.** Aerobic stability of the organism
- **E.** Dose, frequency, duration, and route of administration
- **F.** Gram stain results

The correct answer is A,B, C, D, E, and F. ALL of the parameters listed should be considered in antibiotic selection.

Knowledge Review #2

In a disk diffusion susceptibility test, the disk with the largest inhibition zone diameter indicates that particular antibiotic that should be chosen for the best chance of success against that microorganism.

- ☐ True
- ☐ False

The correct answer is False. The diameter size of the inhibition zone varies by antibiotic and must be compared to the data provided with each impregnated disk. Larger sized zones do not always equal susceptibility and therefore do not indicate clinical success.

Knowledge Review #3

Based on PK/PD profiles, antibiotic agents are commonly divided into groups. Select ALL the correct statements below.

- **A.** Fluoroquinolones are concentration-dependent antibiotics
- **B.** Aminoglycosides are concentration-dependent antibiotics
- **C.** Macrolides are concentration-dependent antibiotics
- **D.** Beta-lactams are time-dependent antibiotics

The correct answers are A, B, and D. A) Fluoroquinolones and B) aminoglycosides are concentration-dependent antibiotics, meaning that the increasing concentrations and AUC will increase efficacy. D) Beta-lactams are time-dependent antibiotics, meaning that the time above MIC is important to achieve clinical effectiveness. C) Some macrolides are time-dependent antibiotics but more data is needed to classify all macrolides.

Knowledge Review #4

Four important PK/PD parameters may be found on a drug label. Match the correct parameter with the correct description.

A.	AUC	**1.**	Maximum plasma drug concentration
B.	CL_{total}	**2.**	An indication of the extent of drug absorption
C.	C_{max}	**3.**	Indicates how fast the drug is cleared from plasma
D.	$T_{1/2}$	**4.**	Time required for the concentration in the plasma to decrease by half

The correct answers are: A – 2; B – 3; C – 1; D – 4.

Knowledge Review #5

A valid VCPR for extra-label use of a drug includes all of the following EXCEPT:

A. A veterinarian has assumed the responsibility for making medical judgments regarding the health of (an) animal(s) and the need for medical treatment
B. The client has agreed to follow the instructions of the veterinarian
C. There is a confirmed diagnosis
D. The practicing veterinarian is readily available for follow-up
E. The veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s)

The correct answer is C. A valid VCPR for ELU does not require a confirmed diagnosis. However, the veterinarian should have sufficient knowledge of the animal(s) to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s).

Knowledge Review #6

Match the following agencies and programs with their correct role in antibiotic regulation or antimicrobial resistance monitoring.

A.	FSIS	**1.**	Program that monitors antimicrobial susceptibility among enteric bacteria from humans, retail meats, and food animals
B.	FDA	**2.**	Establishes the veterinary drug tolerance levels in food products
C.	EPA	**3.**	Establishes pesticide tolerances
D.	FARAD	**4.**	Cooperative project that prevents or mitigates illegal residues of drugs, pesticides, and other chemicals in foods of animal origin
E.	RVIS	**5.**	Ensures that USDA inspected meat, poultry, and egg products are safe and do not contain illegal residues
F.	NARMS	**6.**	National interagency database with information on residue violations in livestock and poultry slaughtered in the US

The correct answers are: A. FSIS – 5; B. FDA – 2; C. EPA – 3; D. FARAD – 4; E. RVIS – 6; F. NARMS – 1.